

Mini-Review on the Cellular Mechanisms of Disease

Fibroblasts, Myofibroblasts, and Wound Contraction

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A clinical case reconstructed by Guido Majno (1975) from the Hippocratic records describes a wrestler who visits the *iatreion* (out-patient clinic) to be treated for a shoulder dislocation. With less invasive procedures no longer working to tighten the dislocation, the clinic adopts the drastic measure of inducing wound contraction by poking a hot needle through the skin of the armpit, and "in this way the cavity, into which the humerus is mostly displaced, is best scarred over and cut off." In Greek medicine circa 400 B.C.E., familiarity with wound contraction after burn injury already was commonplace.

Closure of cutaneous wounds involves three processes: epithelization, connective tissue deposition, and contraction. The contribution of each process varies according to the type of wound. In general, epithelization results in resurfacing of the wound; connective tissue deposition results in replacement of damaged dermis; and contraction brings the margins of open wounds together (Peacock, 1984; Clark, 1988; Mast, 1992). In mammals with loose skin (meaning loosely attached to the underlying tissue layer), wound contraction leads to wound closure with little scarring or loss of function. In humans, whose skin is more firmly attached to underlying tissues, the consequences of contraction are less beneficial, ranging from minimal cosmetic scar in some cases to loss of joint motion or major body deformation in others. Consequently, a distinction has been made between contraction as a normal process of wound closure, and contracture as the abnormal result of the contraction process where significant scarring or loss of function occurs (Hunt and Dunphy, 1979). The pathologic consequences of tissue contraction include a variety of conditions ranging from contracture of the fibrous capsule surrounding breast implants to constriction of hollow organs (e.g., the esophagus) after injury (Skalli and Gabbiani, 1988; Rudolph et al., 1992).

In contemporary cell biology, research on wound contraction focuses on the wound fibroblast. Skin fibroblasts normally are sessile and quiescent, but shortly after cutaneous wounding, they become activated. Activated fibroblasts migrate to the fibronectin-fibrin wound interface, proliferate, and synthesize a new collagen-containing matrix called granulation tissue. Around the same time, wound contraction begins. Once the wound defect is replaced, the expanded fibroblast population stops dividing and regresses and extracellular matrix remodeling commences (Peacock, 1984; Clark, 1993). Despite the importance of wound contraction for wound

healing, the mechanism by which wound fibroblasts exert force on the surrounding extracellular matrix is only beginning to be understood. Moreover, although it has become evident that gene expression and proliferation of these cells is regulated by mechanical force, the mechanotransducers and signaling mechanisms involved remain highly speculative.

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Research carried out in the mid-1950's implicated connective tissue cells in the generation of the tensile force responsible for contraction (Abercrombie et al., 1956). The newly formed granulation tissue was suggested as the "organ of contracture" (Billingham and Russell, 1956). Other studies showed that the force-generating cells were localized at the wound margins rather than in the center of the granulation tissue (Watts et al., 1958). Subsequently, Gabbiani et al. (1972) directly demonstrated that isolated strips of granulation tissue were able to undergo a smooth muscle-like contraction *in vitro* and found that cells within the granulation tissue exhibit some features of smooth muscle cells such as actin filament bundles (stress fibers). These "myo-fibroblasts" were proposed to be responsible for force generation, and their presence has turned out to be a general feature of tissues undergoing contraction (Skalli and Gabbiani, 1987; Rudolph et al., 1992).

Analysis of cytoskeletal markers showed that although myofibroblasts express α -smooth muscle actin, these cells are derived from fibroblasts not smooth muscle cells (Eddy et al., 1988; Darby et al., 1990). During granulation tissue formation, migrating fibroblasts differentiate into myofibroblasts (Darby et al., 1990; Welch et al., 1990). Judging from the presence of fibronexus junctions, myofibroblasts form very tight adhesions to the surrounding fibronectin-rich granulation tissue (Singer et al., 1984; Tomasek and Haaksma, 1991). The timing of the switch from fibroblast to myofibroblast phenotype appears to depend at least in part on the extent to which the wound resists contraction (Darby et al., 1990; Welch et al., 1990). It now seems likely that migrating fibroblasts at the wound margins generate sufficient force to initiate wound contraction. As contraction proceeds and resistance increases, migrating fibroblasts differentiate into myofibroblasts and the actin cytoskeleton becomes organized along the lines of greatest resistance (Petroll et al., 1993).

In Vitro Models of Wound Contraction

Several *in vitro* models of wound contraction have been developed using fibroblasts cultured in collagen or fibrin ma-

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trices. Elsdale and Bard (1972) showed that fibroblasts cultured in collagen matrices acquire tissue-like phenotypic characteristics not typically observed in cells in monolayer culture. They reported that with matrices floating in the culture medium, cell motility resulted in collapse of the matrix into a "dense, opaque body less than one-tenth of the original size." A similar reorganization process was observed when fibroblasts were cultured in fibrin (Niewiarowski and Goldstein, 1973). Subsequently, Bell et al. (1979) began studying collagen-matrix reorganization as an *in vitro* model of wound contraction.

Fig. 1 illustrates three common variations of the *in vitro* collagen matrix contraction model. These are: (I) floating matrix contraction (reduction in diameter); (II) anchored matrix contraction (reduction in height); and (III) stress relaxation. These models differ markedly in their mechanical features. In I, tension is distributed isotropically. In II, tension is distributed anisotropically. In III, mechanical stress develops during the period when the matrix is anchored and then stress dissipates after the matrix is released. (In engineering jargon this process would be called strain recovery rather than stress relaxation.) The next two sections will focus on contraction of floating vs. anchored matrices. Subsequently, stress relaxation will be discussed.

Contraction of Floating vs. Anchored Collagen Matrices

Current evidence suggests that contraction of floating vs. anchored collagen matrices is similar with regard to tractional remodeling, integrins, and extracellular factors (see below). The major difference is in the end result. Contraction of a floating collagen matrix results in a mechanically relaxed tissue, while contraction of an anchored collagen matrix results in a stressed tissue.

Harris et al. (1981) showed that contraction occurs as a consequence of motile activity by cells trying to migrate through the matrix. This process was called "tractional remodeling" to distinguish it from a smooth muscle-like contraction. That is, contraction occurs as fibroblasts spread and elongate, not as already elongated cells retract their extensions (Grinnell and Lamke, 1984). Cells reorganize proximally located collagen fibrils first and subsequently the rest of the matrix as tractional forces are propagated throughout the continuous, intertwined collagen fibril network (Guidry and Grinnell, 1987).

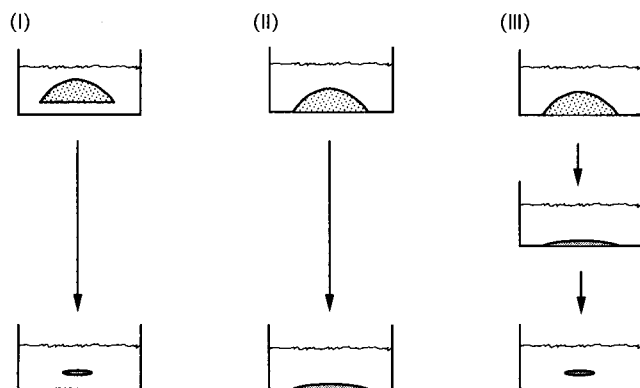


Figure 1.

Adhesive interactions between cells and collagen required for contraction are mediated by $\alpha 2\beta 1$ integrins (Schiroy et al., 1991; Klein et al., 1991). Tensile force was found to depend on an intact actin cytoskeleton (Bell et al., 1979; Bellows et al., 1982; Guidry and Grinnell, 1985) and myosin light chain kinase (MLCK) activity (Ehrlich and Griswold, 1984; Van Bockxmeer et al., 1984; Ehrlich et al., 1991).

Collagen matrix contraction requires serum (Steinberg et al., 1980; Guidry and Grinnell, 1985), which indicates that cell contractility can be regulated by extracellular factors. The activity of serum can be replaced or enhanced by purified growth factors. For instance, transforming growth factor β (TGF- β) stimulates contraction of both floating and anchored collagen matrices (Montesano and Orci, 1988; Finesmith et al., 1990; Fukamizu and Grinnell, 1990). TGF- β also has been reported to promote fibroblast differentiation into myofibroblasts (Ronnov-Jessen and Petersen, 1993; Desmouli et al., 1993). Platelet derived growth factor (PDGF) stimulates matrix contraction (Clark et al., 1989; Gullberg et al., 1990), but by a mechanism independent of TGF- β (Tingstrom et al., 1992). Factors that inhibit matrix contraction include fibroblast growth factor (Finesmith et al., 1990; Dubertret et al., 1991) and interferon γ (Gillery et al., 1992). In general, the downstream signaling mechanisms by which growth factors regulate matrix contraction are unknown, but protein kinase C probably is involved (Danowski and Harris, 1988; Guidry, 1992).

Collagen Matrix Contraction and the Cell Phenotype

The fibroblast phenotype that develops as a consequence of collagen matrix contraction differs dramatically depending on whether the matrices are floating or anchored. Contraction of floating collagen matrices gives rise to a mechanically relaxed tissue whose cells have morphological and proliferative features resembling dermis, whereas anchored matrices develop into a stressed tissue resembling granulation tissue. Strain gauge measurements have shown that the force exerted by fibroblasts in anchored collagen matrices is comparable with that generated in contracting skin wounds or during tooth eruption (Kasugai et al., 1990; Delvoye et al., 1991; Kolodney and Wysolmerski, 1992).

In floating collagen matrices, fibroblasts develop stellate morphology with long processes and a cytoskeletal meshwork (Bell et al., 1979; Bellows et al., 1981). In marked contrast, cells in anchored matrices become bipolar and orient along lines of tension (Stopak and Harris, 1982; Bellows et al., 1982). Cells develop prominent stress fibers and fibronexus junctions and resemble myofibroblasts (Farsi and Aubin, 1984; Mochitate et al., 1991; Tomasek et al., 1992). Therefore, fibroblasts can contract a tissue matrix *in vitro* without differentiating into myofibroblasts, and the appearance of myofibroblasts correlates with development of stress in the matrix.

Fibroblasts in floating vs. anchored collagen matrices show profound differences in cell proliferation. After contraction of floating collagen matrices, there is a marked decline in cellular DNA synthesis (Sarber et al., 1981; Nishiyama et al., 1989; Nakagawa et al., 1989a). The cells become arrested in G₀ (Kono et al., 1990), and cell regression begins (Nakagawa et al., 1989b). Cells in anchored matrices, on the other hand, continue to synthesize DNA and increase in cell number (Nishiyama et al., 1989; Nakagawa et al., 1989a,b).

Also, subjecting fibroblasts in floating collagen matrices to external stress results in increased cell growth (Jain et al., 1990).

The low proliferative capacity of fibroblasts in floating collagen matrices appears to reflect decreased responsiveness of the cells to growth factors (Nakagawa et al., 1989a; Nishiyama et al., 1990, 1991). Recent studies have shown that PDGF receptors on fibroblasts in floating collagen matrices lose their capacity to autophosphorylate in response to PDGF (Lin and Grinnell, 1993).

In addition to changes in cell proliferation, fibroblasts in floating collagen matrices also show decreased collagen biosynthesis and increased release of collagenase compared with cells in anchored matrices (Nusgens et al., 1984; Unemori and Werb, 1986; Paye et al., 1987; Fukamizu and Grinnell, 1990). Changes in collagen and collagenase biosynthesis by fibroblasts in floating matrices depend on transcriptional as well as posttranscriptional mechanisms (Mauch et al., 1988; Lambert et al., 1992; Eckes et al., 1993). These findings suggest that mechanical organization of the tissue can regulate extracellular matrix biosynthesis and remodeling as well as cell proliferation.

Stress Relaxation

If floating collagen matrices resemble dermis and anchored matrices resemble granulation tissue, then stress relaxation represents the transition from granulation tissue to dermis (or scar), albeit on a time scale that is markedly sped up. Unlike the slow (hours to days) contraction of floating or anchored collagen matrices, contraction of stressed collagen matrices occurs in minutes. The fibroblasts themselves contract as indicated by the retraction of cell pseudopodia and collapse of actin filament bundles (Mochitate et al., 1991; Tomasek et al., 1992). In this case, the mechanism appears to involve a smooth muscle-like contraction rather than tractional remodeling. Intact stress fibers are required and the process is regulated by serum factors (Tomasek et al., 1992). Recently, thrombin has been found to substitute for serum and shown to stimulate myosin light chain phosphorylation (Kolodny and Elson, 1993) through a G-protein regulated pathway (Pilcher, B. K., and J. J. Tomasek. 1993. *Mol. Biol. Cell.* 4:300a).

Accompanying stress relaxation, fibroblasts show transient ectocytosis of annexin-containing vesicles (Lee et al., 1993), release of cell surface fibronectin (Mochitate et al., 1991), and inactivation of PDGF receptors (Lin and Grinnell, 1993). Cell proliferation and collagen synthesis decline rapidly as the cells switch from an activated to resting phenotype (Iwig et al., 1981; Mochitate et al., 1991). The signaling mechanisms controlling these different events have yet to be determined, but one of the earliest fibroblast responses to stress relaxation (5–10 min) is activation of a cyclic AMP/protein kinase A signaling pathway (He, J., and F. Grinnell. 1993. *Mol. Biol. Cell.* 4:364a).

Changes in cell proliferation and biosynthetic activity after stress relaxation provide insight into the possible mechanism of myofibroblast disappearance at the end of wound healing. As long as the tissue is under mechanical stress, cell proliferation and biosynthetic activity will persist. Once mechanical stress is relieved, usually by a combination of wound contraction and biosynthetic activity, cells will switch to a non-proliferative phenotype and begin to regress even

in the continued presence of growth factors. This view is consistent with commonplace surgical experience that increased skin tension contributes to increased scarring (Arem et al., 1976; Burgess et al., 1990). Moreover, it helps explain the role of external pressure in reducing wound contracture (Larson et al., 1971; Rockwell et al., 1989).

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